

Emerging incidence of candidemia in neonatal intensive care unit and sick newborn care unit in a tertiary care hospital of Eastern India

Nabamita Chaudhury¹, Monalisa Majumdar^{2*}, Ashok Dutta³, Swarnadeep Das⁴, Tapajyoti Mukherjee⁵, Paulami Ghosh⁶, Purbasha Ghosh⁷

¹Assistant Professor, Department of Microbiology, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal, India

²Professor & Head, Department of Microbiology, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal, India

³Professor & Head, Department of Paediatrics, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal, India

⁴B. Sc Biotechnology, Burdwan Institute of Management & Computer Science, Burdwan University, Dewan Dighi, Katwa Rd, Bardhaman, West Bengal, India

⁵Assistant Professor, Department of Microbiology, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal, India

⁶Senior Resident, Department of Microbiology, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal, India

⁷Assistant Professor, Department of Microbiology, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal, India

Received: 15-06-2020 / Revised: 14-08-2020 / Accepted: 22-08-2020

Abstract

Background: *Candida* blood stream infection (BSI) is an important cause of sepsis and sepsis-related mortality. Common risk factors for *Candida* BSI include very low birth weight, central vascular catheterization (CVC), use of broad-spectrum antibiotics, endotracheal intubation, and prolonged hospital stay. Although *C. albicans* accounts for *Candida* BSI among infants, but recent studies have detected a shift towards non-albicans *Candida* (NAC) species.

Aims & Objectives: To isolate and identify different species of candida from blood samples. To find out the antifungal sensitivity pattern of the fungus isolated. To identify various risk factors associated with Candidemia in patient admitted in critical care unit. **Methods:** BACT/ALERT 3D Paediatric bottle was used for fungal blood culture. Inoculation on Blood agar and Sabourauds dextrose agar (SDA) was made from the culture positive bottles. After the growth obtained from SDA, Gram staining, Germ tube test, CHROM agar Candida Medium and Sugar fermentation and biochemical Test kits (KB006 Hi Candida Identification Kit) were used for identification of various *Candida* Spp. Anti fungal susceptibility test was carried out by Kirby-Bauer disc diffusion method.

Results: Out of 84 different species of *Candida*, *C. albicans* were the highest number (32.14%), followed by 23.81% of *C. tropicalis*, 21.42% *C. parapsilosis*. Susceptibility for voriconazole, fluconazole and amphotericin B was 85.71%, 75% and 64.28%, respectively. NAC (57 isolates) were more resistant to azole group of antifungal, especially commonly used antifungal like fluconazole (45.6%). **Conclusion:** Candidemia is a significant problem in Pediatrics age group patients, especially in NICU and SNCU. A gradual but significant epidemiological shift to higher isolation of NCA is being noticed.

Keywords: Paediatric, *C. albicans*, Non-albicans *Candida* (NAC), Blood stream infection (BSI), Anti-fungal susceptibility test, Resistance, Azole group

This is an Open Access article that uses a fund-ing model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

*Correspondence

Prof. Monalisa Majumdar

Professor & Head, Department of Microbiology, Burdwan Medical College and Hospital, Purba Bardhaman, West Bengal, India. E-mail: drmonalisa92@gmail.com

Introduction

Candidemia (presence of yeasts like fungus in blood) is caused by *Candida* species. *Candida* blood stream infection (BSI) is an important cause of sepsis and sepsis-related mortality[1]. Common risk factors for *Candida* BSI include prematurity and very low birth weight (VLBW), central vascular catheterization, parenteral nutrition, use of broad-spectrum antibiotics, H₂ blockers and corticosteroids, endotracheal intubation, and prolonged hospital stay[1,2]. Although *C. albicans* accounts for 45–55% of *Candida* BSI among infants[1,2,3]. Recent studies have detected a shift towards non-*albicans* *Candida* (NAC) species, which are often associated with high mortality and poor antifungal susceptibility [4,5,6,7]. Among these species, *C. albicans* is still the most common pathogen in spite of its dwindling share. The isolation rates of NCA, other than *C. albicans* vary according to the features (age, underlying diseases, hospitalization ward, etc) of patient population. To illustrate, *C. Parapsilosis* causes 30% of the candidemia cases among newborns[8]. Although there are over 150 *Candida* species in nature, only 15 of them are human pathogens. In the last 20 years, a change has been observed in the rates of *Candida* species isolated from patients with candidiasis. The incidence of *C. albicans* has decreased, while that of the non-*albicans* *Candida* has increased. Although this change has multiple causes, the foremost of these are fluconazole use and the increasing popularity of venous catheters[8,9]. The scope of candidiasis covers a wide range of diseases from more superficial and milder clinical manifestations such as esophageal or oropharyngeal candidiasis to serious infections including blood stream infections (BSIs) and disseminated candidiasis. Currently, there are more than 150 known species of *Candida* that have been identified. Although the isolation frequencies may vary, in the last 20–30 years, it has been determined that in 95% of infections, the pathogens involved are *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*[8]. According to the data provided by the Centers for Diseases Control and Prevention (CDC) and the National Healthcare Safety Network, *Candida* species are ranked fifth among hospital-acquired pathogens and fourth among BSI pathogens[8-11]. According to the results of the SENTRY Antimicrobial Surveillance Program, 1,354 infection episodes related to *Candida* species were detected between 2008 and 2009 and 36.5% of these were community-acquired. Community-acquired candidemia was found to be significantly higher in North America (63.5%) than in Europe (22.4%)[12].

The present study was undertaken to assess the species distribution, susceptibility pattern, risk factors and outcome of neonates developing *Candida* BSI during hospital stay.

Materials and Methods

This was a prospective study which was conducted at the Department of Microbiology and Paediatrics at Burdwan Medical College and Hospital over a 2 years of tenure (January 2018 to December 2019). Permission of the Institutional Ethics Committee was obtained before commencement of the study. A total 801 blood samples of clinically diagnosed sepsis patients' was collected in BACT/ALERT 3D SYSTEM Paediatrics bottles, from Sick neonatal care unit (SNCU), Paediatric ICU (PICU) and in Neonatal ICU (NICU) are included. Samples from the culture positive bottles were inoculated on blood agar and Sabouraud's dextrose agar (SDA) and incubated at 37°C for 24-48 hours. After the growth obtained from SDA and blood agar media, gram stain were carried out to identify the budding cyst. Germ tube test was carried out to differentiate *Candida albicans* and NCA. Chlamydospore formation was carried out to diagnose different species of *Candida*. Chlamydospores are highly refractive, thick walled cells that are produced under nutrient poor, oxygen limited condition and in low temperature[1,10,18] CHROM agar *Candida* Medium was used for presumptive identification of various *Candida* Spp. This is based on the direct detection of specific enzymatic activities by adding certain substrates of fluorochromes to the media. A subculture will be made from primary isolation media in CHROM agar *Candida* Medium, and it will be incubated at 30°C for 24-48 hours. After 24 -48 hours, colony morphology and colour of the colony will be noted. CHROM agar *Candida* was used to differentiate several *Candida* Spp by colour and morphology. Sugar fermentation and biochemical Test kits (KB006 Hi*Candida* Identification Kit) was carried out. The yeast to be identified was isolated on SDCA. The inoculum was prepared by picking 2-4 well isolated colonies and making homogeneous suspension in 2-3 ml sterile saline Each well of the kit was inoculated with 50µl of the above inoculum by surface inoculation or by stabbing each individual well with a loop full of inoculum. Incubated at 22.5°C ±2.5°C for 24-48 hours.

Anti Fungal Susceptibility Test (AFST)

This was carried out next by the disc diffusion. Using amphotericin-B (100 unit), voriconazole (1µg) and

fluconazole (10ug). Mueller Hinton agar supplemented with 2% glucose and methylene blue dye 0.5µg/ml. The risk factors for candidemia will be identified from the demographic data of the patients or from hospital records for the retrospective arm. Presence of the following factors were considered as risk factors and were matched for association with positive *Candida* culture:

Risk factors for invasive candidiasis for children

- Mechanical ventilation
- Usage of antibiotics with broad spectrum
- Catheter in situ e.g. central line, urinary catheter, central venous catheter
- Prolonged stay in hospital >14 days

Results

Out of 801 blood samples, 47.31% were culture positive. Among which 56.67% were Gram positive cocci, 22.95% were Gram negative bacilli and 21.37% (n=84) were *Candida* isolates. Nearly 64.28% isolates of *Candida* were obtained from NICU, followed by 29.76% were from SNCU, 5.95% were PICU. ICU male patients had outnumbered the females. About 46.42% patients were diagnosed with sepsis with PUO, followed by 21.42% suffering from pneumonia, 14.28% were diagnosed with congenital heart disease. *C. albicans* were the highest numbers of isolate, accounting for 32.14%, next to it was *C. tropicalis* (23.81%), *C. parapsilosis* (21.42%), *C. krusei* (14.28%) and *C. glabrata* (8.33%). Voriconazole revealed 85.71% sensitivity, followed by fluconazole and amphotericin b accounting for 75 % and 64.28% sensitivity rate respectively.

Table 1: Direct detection of different species of *Candida* on the basis of different colour on Chrome agar media

<i>Candida</i> spp	Colour on CHROM agar media
<i>C. albicans</i>	Light green
<i>C. glabrata</i>	Grey to white
<i>C. tropicalis</i>	Steel blue
<i>C. parapsilosis</i>	Off white to cream
<i>C. krusei</i>	Purple, fuzzy

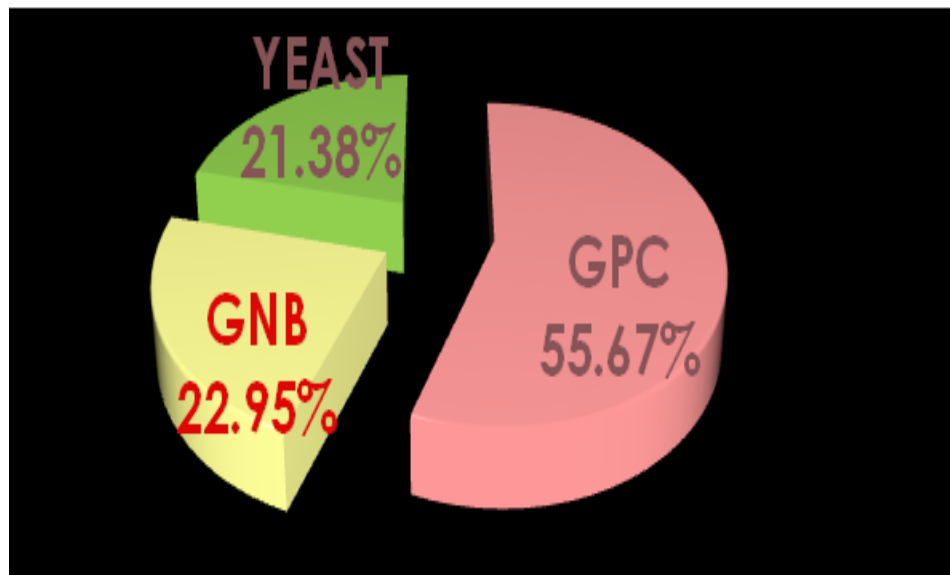


Figure1: Distribution of different culture positive isolates



Figure 2: Chrome agar *Candida* medium

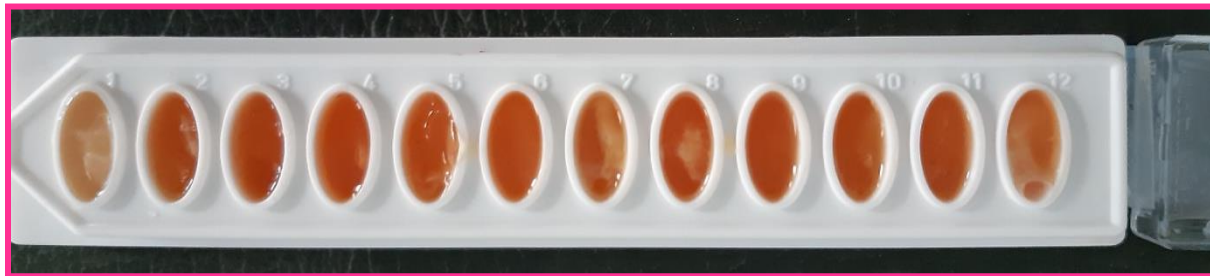


Figure 3: Sugar fermentation and biochemical Test kits (KB006 HiCandida Identification Kit)

Table 2: Interpretation of Sugar fermentation and biochemical Tests by different species of *Candida* by KB006 HiCandida Identification Kit

<i>Candida</i> spp	Urease	Meli bios e	Lact ose	Maltose	Sucro se	Gal ac tose	Cell pb iose	Inosit ol	Xylo se	Dulcit ol	Raffinose	Treha lose
<i>C.albicans</i>	-	-	-	+	-	+	+	-	-	+	-	+
<i>C.glabrata</i>	+	-	-	-	-	-	-	-	-	-	-	-
<i>C.tropicalis</i>	-	-	-	-	-	-	+	-	-	-	-	-
<i>C.parapsilo sis</i>	-	-	-	+	+	+	+	-	-	+	+	+
<i>C.krusei</i>	+	-	-	-	-	-	-	-	-	-	-	-

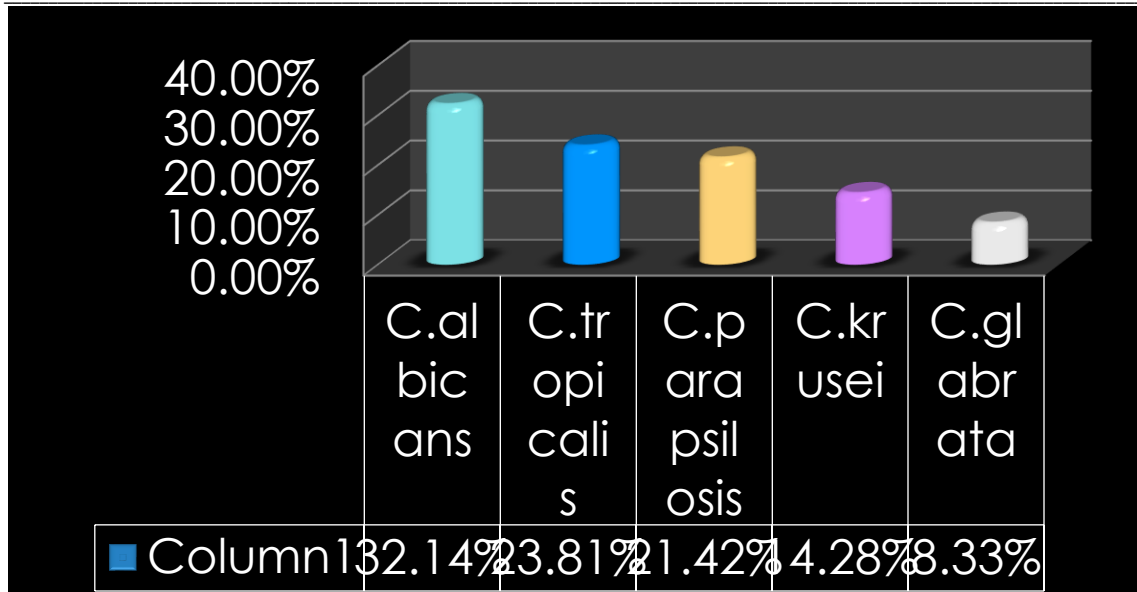


Fig 4: Distribution of different species of *Candida*

Table 3: Distribution of different species of *Candida* according to the risk factors

Sources of infection (Potential risk factors)	<i>Candida albicans</i>	NCA (n=57)
Mechanical ventilation	51.8%	54.4%
Catheter in -situ	51.8%	54.4%
Usages of antibiotics with broad spectrum	37%	66.66%
Prolonged stay in hospital <14 days	81%	82.4%



Figure 5: Antifungal susceptibility test (AFST)

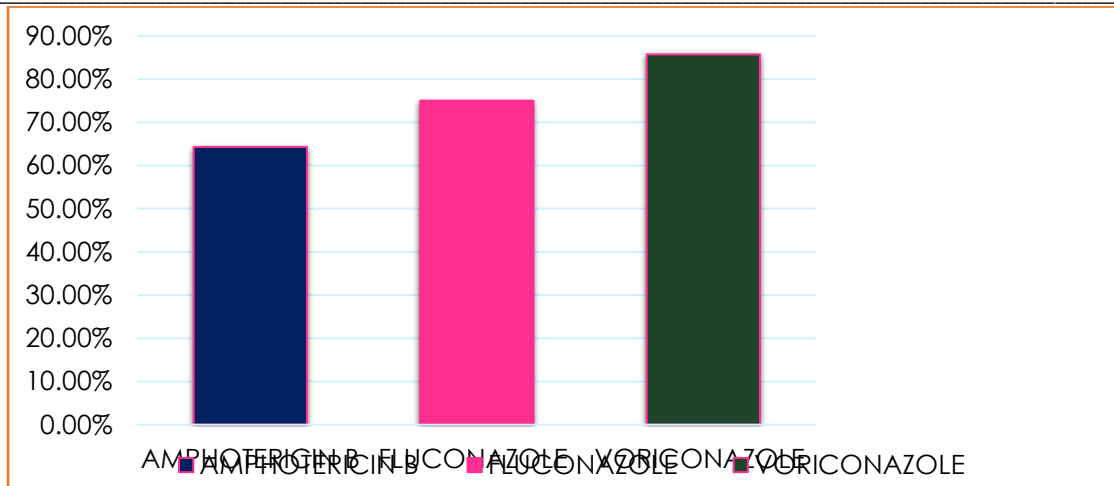


Figure 6: Antifungal susceptibility testing (AFST)

Discussion

Candida BSI is an emerging and life threatening conditions that endangers the critically ill patients admitted at ICU. In the present study the incidence of *Candida* BSI was 10.48% of total clinically suspected patients' of sepsis. *C. albicans* was the most commonly isolated *Candida* species, followed by *C. tropicalis* and *C. parapsilosis*. All total the isolates of NCA was outnumbered the total isolated of *C. albicans* (Fig. 4). This finding was consistent with the results of a study that reported a predominance of non-*C. albicans* species (64% non-*C. albicans* versus 36% *C. albicans*) [13]. Several studies also reported this pattern in contrast to the results of older published [14,15]. Since the last few decades, the epidemiology of Candidemia has been evolving as there is a progressive shift from a predominance of *Candida albicans* toward a predominance of non-*albicans Candida* spp, especially *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. Emergence of NAC species and their association with higher mortality and longer duration of hospital stay is a cause for concern [16]. This scenario was quite similar with our present study as, we have observed that a higher incidence of candidemia is associated with prolonged hospital stay: more than 14 days (Table 3). The incidence of sepsis and duration of hospital stay were significantly higher in NAC BSI. Catheter in situ and hospital stay >14 days were independent predictors of NAC BSI, in this study. Unlikely, another study by Khairat et al revealed that the most important risk factor was previous exposure to antibiotics for >14 days [13].

The colonization of *Candida* on the skin and on the mucosal membrane is one of the pivotal factors for commencing Candidemia. After the colonization on the skin and mucus membrane, *Candida* disrupt the host's natural epithelial barrier, due to insertion of intravascular catheters or mechanical ventilators or surgery or burns. Candidemia can occur in a hospital setting as a primary Central Line Associated Bloodstream Infection (CLABS I) or secondary bloodstream infection [16]. In our study we have founded that 51.8% of *C. albicans* isolates and 54.4% of NAC were obtained from those patients in whom catheters were in situ (Table 3). It is evident that the BSI due to *Candida* species contributes to mortality is substantial and the targeted and appropriate antifungal therapy can control this menace. In this present study, candidemia was most frequently identified in NICU (64%) and 29.8% were from SNCU. These findings were consistent with another study where 59% were in ICU and in neonates 35% [13]. These statistics depicted that the growing number of isolates of *Candida* creating a problem in ICUs. In the last few decades, antifungal susceptibility testing has become standardized and nowadays has the same role of the antibacterial susceptibility testing in microbiology laboratories. With regards to antifungal susceptibility, 64.28% of isolates were sensitive to Amphotericin B (Fig 6). However, some other studies reported lower incidence of resistance, unlike this study. A study by Khairat et al revealed only 35% isolates were resistance to Amphotericin-B [13]. Similarly a study by Sook-In Jung, et al reported lower incidence of Amphotericin-B resistance [14]. Back to 2005 to 2012, some previous studies even reported no resistance to Amphotericin-B at all [15-17]. This increasing trends of

Amphotericin-B resistance might be due to extensive use of Amphotericin B or judicious use of broad spectrum use of antibiotics or long time presence of catheter-in-situ[13]. The rate of Fluconazole sensitive rate was 75%. However, Voriconazole revealed 85.7% sensitivity rate. Both of these findings of our study were consistent with the result of another study[13]. According to some other study the rate of resistance to fluconazole and voriconazole is pretty much lower[14]. Many studies on the correlation of *in vitro* results with the outcome of patients have been performed, reaching the conclusion that infections caused by resistant strains have worse outcome than those caused by susceptible *Candida* isolates. These studies have allowed the development of interpretative breakpoints for *Candida* spp, the most frequent agents of fungal infections in the world. In summary, antifungal susceptibility tests have become essential tools to guide the treatment of fungal diseases, to know the local and global disease epidemiology, and to identify resistance to antifungals. Prevention of risk factors in susceptible paediatrics age group with early removal of central line or urinary catheter, early reporting of fungal culture till species level of *Candida* and susceptibility testing are necessary for appropriate institution of treatment and better outcome. Frequent empirical use of fluconazole and amphotericin B may be avoided as it may lead to a shift in species distribution and higher antifungal resistance.

Conclusion

Candidemia is a significant problem in Pediatrics age group patients, especially in NICU and SNCU. A gradual but significant epidemiological shift to higher isolation of NCA is being noticed. It is imperative that routine screening of *Candida* isolates to the species level is essential and could assist clinicians in promoting adoption of important prophylactic and treatment guidelines for its improved management.

References

- Colombo AL, Guimarães T, Silva LR, de Almeida Monfardini LP, Cunha AK, Rady P, et al. Prospective observational study of candidemia in São Paulo, Brazil: incidence rate, epidemiology and predictors of mortality. *Infect Control Hosp Epidemiol.* 2007; 28(5):570-6.
- Yamamoto M, Takakura S, Hotta G, Matsumura Y, Matsushima A, Nagao M, et al. Clinical characteristics and risk factors of non-*Candida* fungaemia. *BMC Infect Dis.* 2013; 13:247-53.
- Dóczy I, Petó Z, Fodor E, Bereczki L, Nagy E, Hajdú E. Evaluation of fungaemia infections in a Hungarian university hospital between 1996 and 2009. *Acta Microbiol Immunol Hung.* 2012; 59(1):29-41.
- Rosas RC, Salomão R, da Matta DA, Lopes HV, Pignatari AC, Colombo AL. Bloodstream infections in late-stage acquired immunodeficiency syndrome patients evaluated by a lysis centrifugation system. *Mem Inst Oswaldo Cruz.* 2003; 98(4):529-32.
- Anunnatsiri S, Chetchotisakd P, Mootsikapun P. Fungemia in non-HIV-infected patients: a five-year review. *Int J Infect Dis.* 2009; 13(1):90-6.
- Asmundsdóttir LR, Erlendsdóttir H, Gottfredsson M. Increasing incidence of candidemia: results from a 20-year nationwide study in Iceland. *J Clin Microbiol.* 2002; 40(9):3489-92.
- Almirante B, Rodríguez D, Park BJ, Cuenca-Estrella M, Planes AM, Almela M, et al.; Barcelona Candidemia Project Study Group. Epidemiology and predictors of mortality in cases of *Candida* bloodstream infection: results from population-based surveillance, Barcelona, Spain, from 2002 to 2003. *J Clin Microbiol.* 2005; 43(4):1829-35.
- Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent problem. *Clin Microbiol Rev.* 2007;20(1):133-163.
- Pappas PG. Invasive candidiasis. *Infect Dis Clin North Am.* 2006;20(3):485-506.
- Sievert DM, Ricks P, Edwards JR, et al. National Healthcare Safety Network (NHSN) Team and Participating NHSN Facilities Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention 2009-2010. *Infect Control Hosp Epidemiol.* 2013;34(1):1-14.
- Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis.* 2004;39(3):309-317.
- Pfaller MA, Moet GJ, Messer SA, Jones RN, Castenheire M. *Candida* bloodstream infections: Comparison of species distributions and antifungal resistance patterns in community-onset and nosocomial isolates in the SENTRY antimicrobial surveillance program, 2008-2009. *Antimicrob Agents Chemother.* 2011;55(2):561-566.

13. Khairat SM, Sayed AM, Nabih M, Soliman NS, Hassan YM. Prevalence of *Candida* blood stream infections among children in tertiary care hospital: detection of species and antifungal susceptibility. *Infect Drug Resist.* 2019;12:2409-2416.
14. Jung SI, Shin JH, Choi HJ, et al. Antifungal susceptibility to amphotericin B, fluconazole, voriconazole, and flucytosine in *Candida* bloodstream isolates from 15 tertiary hospitals in Korea. *Ann Lab Med.* 2012;32(6):426-428.
15. Caggiano G, Coretti C, Bartolomeo N, Lovero G, De Giglio O, Montagna MT. *Candida* bloodstream infections in Italy: changing epidemiology during 16 years of surveillance. *Biomed Res Int.* 2015; 2015:256580.
16. Capoor MR, Nair D, Deb M, Verma PK, Srivastava L, Aggarwal P. Emergence of non-albicans *Candida* species and antifungal resistance in tertiary care hospital. *Jpn J Infect Dis.* 2005;58(6):344–348.
17. Mota AJ, Graziella N, Back-Brito GN, Nobrega FG. Molecular identification of *Pichiaguillier mondii*, *Debaryomyces hansenii* and *Candida palmioleophila*. *Genet Mol Biol.* 2012;35:122–125.

Source of Support: Nil

Conflict of Interest: Nil